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Wind-tunnel assays to determine the effect of minor pheromone components on the responses of male spruce budworm

C.J. Sanders

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FORESTRY CANADA, ONTARIO REGION

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WIND-TUNNEL ASSAYS TO DETERMINE THE EFFECT OF MINOR PHEROMONE COMPONENTS ON THE RESPONSES OF MALE SPRUCE BUDWORM

C. J. SANDERS

FORESTRY CANADA

ONTARIO REGION

GREAT LAKES FORESTRY CENTRE

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ABSTRACT

Bioassays of the sexual behavior of male spruce budworm (*Choristoneura fumiferana* [Clem.]) were carried out in a wind-tunnel in an attempt to identify minor pheromone components that might improve male responses to the 95:5 blend of the major pheromone components, the unsaturated aldehydes E- and Z-11-tetradecenal. Filter paper and rubber septa were used to dispense the pheromone. The septa were placed on cones of filter paper, which permitted the assay of courtship and copulatory attempts in addition to activation and in-flight responses.

Addition of E-11-tetradecenyl acetate, which is present in the female-emitted pheromone at less than 1% of the concentration of the aldehydes, reduced all levels of response at concentrations of 10% or greater. Addition of the saturated aldehyde tetradecanal, which is present in the female-emitted pheromone at a concentration of 1-2% of that of the unsaturated aldehydes, had no significant effect on responses, either alone or in combination with the acetate.

Addition of hexadecanal had a slightly inhibitory affect on male behavior, but addition of E- or Z-11-hexadecenal had no effect.

None of the blends at any of the ratios or concentrations tested evoked responses as high as those evoked by calling, virgin female moths. It is therefore hypothesized that additional unidentified compounds are present in the natural pheromone.

RÉSUMÉ

Des essais biologiques portant sur le comportement sexuel de la tordeuse mâle des bourgeons de l'épinette (*Choristoneura fumiferana* [Clem.]) ont été effectués dans une soufflerie afin de déterminer les constituants mineurs de la phéromone qui pourraient améliorer la réponse des mâles au mélange 95:5 des principaux constituants de la phéromone, les aldéhydes tétradécényliques non saturés E-11 et Z-11. Du papier-filtre et des cloisons en caoutchouc ont été utilisés pour distribuer la phéromone. Les cloisons ont été placées sur des cônes de papier-filtre, ce qui a permis les essais de parades nuptiales et de copulation en plus de l'activation des réponses en cours de vol.

L'addition d'acétate de E-11-tétradécényle, qui est présent dans la phéromone produite par la femelle à une concentration inférieure à 1% de celle des aldéhydes, a eu pour effet de réduire tous les niveaux de réponse à des concentrations de 10% ou plus. L'addition d'aldéhyde

myristique saturé, qui est présent dans la phéromone produite par la femmelle à une concentration de 1 à 2% de celle des aldéhydes saturés, n'a pas eu d'effet important sur les réponses, même lorsqu'on a ajouté de l'acétate de E-11 tétradécényle.

L'addition d'aldéhyde myristique a eu peu d'effets inhibitifs sur le comportement des mâles, et les aldéhydes hexadécényliques E-11 et Z-11 n'ont eu aucun effet.

Aucun des mélanges ni aucun des rapports de concentrations testés n'ont suscité de réponses aussi grandes que celles obtenues par l'appel de la tordeuse vierge. On suppose donc que d'autres substances non identifiées sont présentes dans la phéromone naturelle.

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INTRODUCTION

The major component of the sex pheromone of the spruce budworm (*Choristoneura fumiferana* [Clem.]) was identified by Weatherston et al. (1971) as E-11-tetradecenal (E11-14AL). Subsequently, Z-11-tetradecenal was shown to be an additional important component, present at a concentration of about 4-5% in the emitted pheromone, and maximum numbers of male spruce budworm are captured in traps baited with a mixture of 95-98% of the E isomer and 2-5% of the Z isomer of the aldehyde (Sanders and Weatherston 1976, Silk et al. 1980). A 95:5 blend is now used as the standard lure for spruce budworm male moths and this blend will be referred to as E/Z11-14AL throughout this paper.

Assays of a wide range of concentrations of the 95/5 blend have been carried out in wind tunnels for various types of dispensers: polyvinyl chloride (PVC) pellets (Sanders et al. 1981), rubber septa (Silk and Kuenen 1986; Sanders, manuscript in preparation), and filter paper (Sanders, manuscript in preparation). However, none of these assays has evoked levels of response equal to those in response to a calling female moth.

Such limitations in the synthetic pheromone blend could have important consequences in its use for mating disruption. As concluded by Minks and Cardé (1988), the available evidence indicates that maximum disruption of mating behavior is achieved by using the complete, natural, pheromone blend. Considerable effort has therefore gone into identifying additional compounds in the female-emitted pheromone that might enhance the effect of E/Z11-14AL.

Silk et al. (1980) reported the presence of two minor components, the saturated aldehyde tetradecanal [SAT-14AL], which is present at a concentration of 2% of that of E/Z11-14AL, and E-11-tetradecenyl acetate [E11-14AC], which is a precursor of the aldehyde pheromone (Morse and Meighen 1986, Wolf and Roelofs 1987) and is present at less than 1% of that of the aldehydes. The role of these two components in the pheromone blend has not been fully resolved. When emitted from the same trap as the 95:5 blend of the unsaturated aldehydes, E11-14AC results in reduced catches (Sanders et al. 1972, Sanders 1976, Alford et al. 1983). Because E11-14AC is a major component of the pheromone of the jack-pine budworm (*Choristoneura pinus pinus* Free.), the sympatric sibling species, it is probable that the 'inhibitory' effect of E11-14AC is a mechanism involved in maintaining the integrity of the two species (Silk et al. 1985). The role of the saturated aldehyde SAT-14AL is more enigmatic. Alford et al. (1983) and Sanders (1984) showed that the presence of SAT-14AL in combination with E11-14AC offsets the inhibitory effect of the acetate. Alford et al. (1983), Sanders (1984, 1985) and Silk and Kuenen (1986) have all shown that, in combination with E/Z11-14AL, E11-14AC increases the proportion of males that reach and contact the pheromone source; Grant (1987) has shown that its presence resulted in males attempting to copu-

late with rubber septa sooner and over a more prolonged period. There is, then, a body of data that indicates that the addition of SAT-14AL increases the numbers of males that contact a pheromone source and the numbers that attempt to copulate with the source. However, there are inconsistencies in the data, and in all experiments in which the comparison has been made, no blend of E/Z11-14AL evokes a level of response equal to that evoked by virgin, calling females.

More recently, Silk and Kuenen (1988) deduced the presence of hexadecenals (16ALs) from chemical precursors in the emitted pheromone, and Z-11-hexadecenal (Z11-16AL) has been identified subsequently in the emitted pheromone at concentrations of less than 1% of those of the 14ALs (P.J. Silk, New Brunswick Research and Productivity Council, personal communication).

In an attempt to resolve further the role of SAT-14AL, E11-14AC and the 16ALs in spruce budworm sexual behavior, and to develop a more effective blend for use in disruption of mating, wind-tunnel bioassays were carried out with a range of concentrations and blends of SAT-14AL, E11-14AC, SAT-16AL, E11-16AL, and Z11-16AL added to the basic 95:5 blend of E/Z11-14AL. In-flight behavior and the behavior of males after they had landed and contacted the pheromone source were evaluated.

With minor exceptions, none of the added compounds produced an improvement in responses over the basic E/Z11-14AL and certainly did not enhance responses to a level comparable with that evoked by a virgin female moth. In that sense, the results are largely negative. However, because of the economic significance of this insect and the major effort directed at developing its pheromone as a control agent, these results are reported here.

MATERIALS AND METHODS

Insects

The insects used in these experiments were laboratory-reared stock maintained at the Forest Pest Management Institute (FPMI) and Forestry Canada, Ontario Region (FCOR), Sault Ste. Marie, Ontario. The stock originated from insects collected in Ontario, and is supplemented annually by field-collected insects. Larvae were reared on artificial diet (Grisdale 1970) at 21°C and 70% relative humidity. Sexes were separated at the pupal stage, and moths were kept in separate cages under a 17:7 light/dark cycle. Female moths were kept under an advanced light/dark cycle (lights off at 15:00 hr), which caused them to start calling by 14:00 hr. Male moths were kept on a more natural cycle, with lights off at 20:30 hr. Assays were carried out between 14:00 and 16:00 hr, as in previous investigations in this laboratory. This time is 3 to 4 hr before the onset of female calling, when the basic level of activity among males in the absence of pheromone is low (Sanders 1971). As a result, when male moths are activated by the pheromone, the action is

pronounced and is not confounded by the presence of individual males that are already showing a high level of activity.

Chemicals

The compounds tested, E11-14AL, Z11-14AL, E11-14AC, SAT-14AL, SAT-16AL, E11-16AL and Z11-16AL, were obtained from the New Brunswick Research and Productivity Council. Solutions were prepared in hexane and were checked for purity and accuracy of concentrations by gas chromatograph (GC) analysis on two 30-m capillary columns, HP 101 (Hewlett Packard) and SP 2340 (Supelco). All solutions were >99% pure, and the aldehydes contained no detectable acetates or alcohols. Assays were carried out with filter paper and rubber septa as dispensers. The filter paper dispensers were 2.5- x 2.5-cm squares cut from Whatman #3 paper and were loaded with 10-µl aliquots of the test solutions. The rubber septa were obtained from A.H. Thomas and Company, Philadelphia (catalogue #1780JD07). Extractions were performed on septa before use to remove compounds that might react with the aldehydes; the technique described by Steck et al. (1979) was used. Septa were then loaded with 100-µl aliquots of the test solutions.

Experimental Procedures

All experiments were carried out in the wind tunnel described in Sanders (1982). All pheromone sources (female moths, filter paper or rubber septa) were fastened to a wire stand that positioned them in the center of the upwind screen. Female moths, 1-2 days old, were housed individually in screen cages, 3 cm in diameter x 2.5 cm long. Two cages, taped end to end, were used for assays in case one female was not calling at any given time. Filter paper dispensers were prepared by pipetting 10 µl of the test solution onto a square of filter paper, which was then kept for 5 min in a fume hood to allow the solvent to evaporate. The filter paper was then pinned vertically to the stand, and assays were completed during the following 15 min. Rubber septa were loaded with 100 µl of test solution, pipetted into the open end of the septum. Septa were left in a fume hood for 48 hr before assays. Between assays they were kept refrigerated. For the assays the septa were pinned onto filter paper cones, constructed from 7-cm-diameter #3 Whatman paper, with a vertical axis of 2.5 cm (Fig. 1). These provided an arena for evaluation of the close-range behavior of the males. Septa were oriented with the open end downwind to mimic the position of a female moth. To assess the response of male moths to septa in the presence of natural female pheromone, a floor was fitted to the cone and two virgin female moths were placed inside. Two 10-mm-diameter holes were then cut and covered with screening, one in the floor and one in the top surface of the cone where the septum was to be pinned. Airflow over the cone drew air up through the compartment in which the female moths were housed and over the septum.

Female moths were placed in position at 11:00 hr, 3 hr before the start of a series of assays, to allow for them to settle down and start calling. Males were transferred individually to screen cages, 3 cm in diameter x 7 cm long, at 11:00 hr and were placed on a bench to one side of the downwind end of the tunnel under a light intensity of 30 lux, similar to that in the center of the wind tunnel.

Behavioral assays were carried out by first moving each male, in its cage, into the tunnel, off to one side and out of the pheromone plume; 0.25 min was allowed for the male to acclimate. The cage was then moved laterally into the pheromone plume about 180 cm downwind from the source. When the male began to move, the screen cover was removed, and the subsequent behavior of the moth was recorded. Times at which the following behavioral steps occurred were recorded after the motns were moved into the pheromone plume: wing-fanning, flying, locking onto the pheromone plume at a point 130 cm from the source (about 50 cm upwind from the release point), reaching a point 30 cm from the source, and landing on or adjacent to the source. The length of time it took a male to fly from the 130-cm mark to the 30-cm mark was used to calculate its flight speed upwind. When septa were used, the following additional information was recorded: the time of the first copulatory attempt, the number of copulatory attempts in a 3-min period and the number of 0.25-min intervals (maximum = 12) in a 3-min period in which the male fanned his wings. This latter figure was used as an index of the intensity of the male's sexual activity. All times were recorded to the nearest 0.01 min.

For measurement of sustained flight, the protocols described in Sanders et al. (1981) were followed. Males were released, as above, in the pheromone plume. As they proceeded upwind, the patterned ceiling of the tunnel was moved backwards above the flying moths. By adjusting the speed of the ceiling, the moths were held in flight in the center of the tunnel. The duration of flight was recorded from the moment the moth locked on until it veered off and landed on the side of the tunnel or on the floor.

Data were analyzed as follows. The numbers of moths that responded to the different treatments were analyzed for differences by means of the G test (Sokal and Rohlf 1981), and the transformation n+1 was used to avoid counts of zero. Times were transformed to logarithms to normalize the distributions and then were subjected to analysis of variance and, where appropriate, to Tukey's procedure for multiple comparisons (Steel and Torrie 1980).

RESULTS

E/Z11-14AL Plus E11-14AC

Initial assays of mixtures of E/Z11-14AL and E11-14AC were carried out with filter paper as the dispenser. Two concentrations of

E/Z11-14AL were assayed, 0.1 and 0.01 μg, in combination with E11-14AC at concentrations of 0.1, 1, 10 and 100% of the E/Z11-14AL concentrations (Table 1). At concentrations of 10% or higher, the numbers of males that locked on, flew upwind, and reached the pheromone source all decreased.

Additional assays were carried out with septa mounted on filter-paper cones (Fig. 1) to assess the effects of E11-14AC on close-range and

Table 1. Responses of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with E11-14AC on filter paper. The 0.01- and 0.1-μg E/Z11-14AL series were analyzed separately.

E/Z11-14AL (μg)	E11-14AC (as % of E/Z11-14AL)	n	Proportion (%) of male moths that:[a] Locked on	Came within 30 cm of source	Reached source	n	Flight speed (cm/sec)
0.01	0	36	75 a	96 a	61	25	11.6
"	0.1	36	86 a	91 ab	64	25	11.7
"	1	36	92 a	75 ab	72	15	10.43
"	10	36	64 a	69 ab	50	-	-
"	100	36	8 b	33 b	0	-	-
0.1	0	30	70 a	76	69	24	12.0
"	0.1	30	63 ab	74	86	14	10.8
"	1	30	70 ab	95	85	31	12.5
"	10	30	33 bc	60	33	8	15.9
"	100	24	8 c	100	50	-	-

Note: Numbers in the same column that are followed by different letters are significantly different (P = 0.05).

[a] Percentages were calculated from the numbers that reached the previous behavioral stage.

in-flight male behavior. One concentration of E/Z11-14AL was used, 100 µg/septum, which is well within the optimum range (Sanders, manuscript in preparation). Three concentrations of E11-14AC were assayed, 1, 3 and 10% of the concentration of E/Z11-14AL. The addition of 1% E11-14AC led to higher proportions of moths that locked on and flew upwind to the pheromone source, but to a slightly smaller proportion that attempted copulation (Table 2). However, none of the differences was significant. When 10% E11-14AC was added, significantly fewer insects reached the source, and of these, fewer still attempted copulation with the septum. Increasing the concentration of E11-14AC also resulted in significantly slower upwind flight (Table 2).

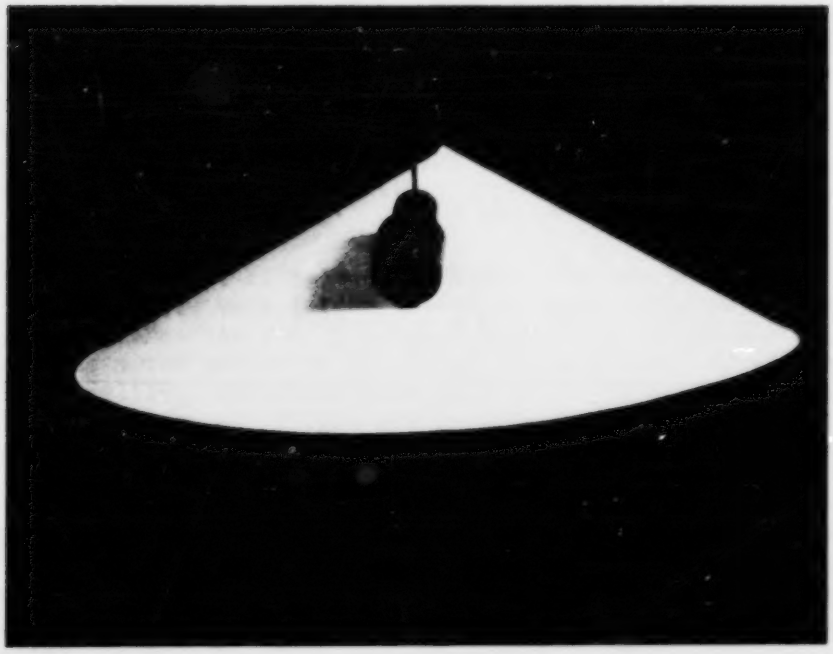

Figure 1. Rubber septum pinned to filter-paper cone for use in wind-tunnel bioassays.

Table 2. Responses of male spruce budworm (n = 50) in a wind tunnel to 100 μg of E/Z11-14AL in combination with E11-14AC loaded in rubber septa.

E11-14AC (as % of E/11-14AL)	Proportion (%) of male moths that:[a] Locked on	Came within 30 cm of source	Reached source	Attempted copulation	n	Flight speed (cm/sec)
0	84 ab	86	92 a	76 a	33	17.0 a
1	94 a	89	100 a	74 a	37	16.5 ab
3	72 b	92	82 ab	52 ab	27	15.2 ab
10	62 b	90	56 b	33 a	15	11.04 b

Note: Numbers in the same column that are followed by different letters are significantly different (P = 0.05).

[a] Percentages were calculated from the numbers that reached the previous behavioral stage.

E/Z11-14AL Plus SAT-14AL

The first assays of mixtures of E/Z11-14AL and SAT-14AL were carried out with filter paper as the pheromone dispenser. Previous experiments have established that the optimum loading of E/Z11-14AL on filter paper is in the range from 0.1 to 10 μg (Sanders, manuscript in preparation). For these experiments, three loadings of E/Z11-14AL were used, 0.0003, 0.001 and 0.003 μg. These suboptimal concentrations were chosen so that any enhancement of response as a result of the SAT-14AL would be detectable. SAT-14AL was added at concentrations of 0.1, 1, 10, 100 and 1000% of that of the E/Z11-14AL concentration. As anticipated from previous experiments (Sanders, manuscript in preparation), the numbers of moths that locked onto the pheromone plumes increased with increasing concentration of E/Z11-14AL. However, within each different level of concentration of E/Z11-14AL, the addition of SAT-14AL had no significant effect on the responses (Table 3).

Experiments were repeated with septa as dispensers to evaluate close-range responses and in-flight behavior. Again, suboptimal concentrations of E/Z11-14AL were used (0.01, 0.1 and 1 μg per septum) to determine if SAT-14AL has a synergistic effect on male response. Concentrations of SAT-14AL assayed were 0, 1, 3, 10 and 30% of those of the E/Z11-14AL. With loadings of 0.01 μg E/Z11-14AL no males locked onto

Table 3. Response of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with SAT-14AL, with filter paper as a dispenser. The three series of E/Z11-14AL concentrations were analyzed separately. Within each series, no differences were significant.

E/Z11-14AL (μg)	SAT-14AL (as % of E/Z11-14AL)	n	Proportion (%) of male moths that:[a] Locked on	Came within 30 cm of source	Reached source	n	Flight speed (cm/sec)
.0003	0	30	7	100	100	-	-
"	1	30	0	-	-	-	-
"	10	30	7	100	100	-	-
"	100	30	7	100	100	-	-
"	1000	30	10	67	100	-	12.5
.001	0	40	35	93	100	13	13.3
"	.1	31	26	100	88	8	13.5
"	1	34	44	87	100	11	13.8
"	10	30	33	90	100	8	12.1
"	100	30	40	92	91	11	13.0
"	1000	31	39	92	100	9	15.7
.003	0	78	63	94	89	17	14.5
"	.1	30	73	95	90	21	15.7
"	1	77	68	94	96	22	16.6
"	10	31	63	100	100	20	15.0
"	100	33	76	92	91	22	16.4
"	1000	30	87	96	92	26	14.5

[a] Percentages were calculated from the numbers that attained the previous behavioral stage.

the septa (Table 4). With loadings of 0.1 and 1.0 μg E/Z11-14AL, an average of 21% and 89%, respectively, of the males locked on (Table 4). The addition of SAT-14AL had no significant effect on the levels of response. With loadings of 0.1 μg E/Z11-14AL too few males flew upwind to the source to permit comparison of flight speed and intensity of sexual activity. At loadings of 1.0 μg E/Z11-14AL, addition of SAT-14AL had no significant effect on the intensity of sexual activity or on flight speed.

Because only a few males were activated at concentrations of 0.01 and 0.1 μg E/Z11-14AL per septum, the numbers of insects available for determining subsequent responses to different concentrations of SAT-14AL were low. Therefore, assays were carried out in which males were first activated by a septum loaded with 1 μg of E/Z11-14AL. (These are referred to hereafter as 'preactivated' males.) The 1-μg septum was placed a short way down the tunnel, downwind from the septum being tested, and when a male locked onto the plume, the 1-μg septum was quickly removed, leaving the male in the plume from the lower-concentration septum. When the 1-μg septum was removed, males immediately started to cast across wind in a manner similar to that of males that have lost a pheromone plume completely. However, in the presence of a 0.1-μg septum, 94% of the males again proceeded upwind after a brief interval; only 14% proceeded upwind to a 0.01-μg septum. The presence of the SAT-14AL had no significant effect on the levels of response, the intensity of sexual activity or flight speed. Males that were preactivated took significantly longer (0.17 vs 0.12 min., $F = 3.34$, $df = 124$) to lock on and to move upwind in response to a septum loaded with 0.1 μg E/Z11-14AL than males that were exposed directly to the 0.1-μg septum (referred to hereafter as 'naive' males). Preactivated males also spent more time casting in the 0.01-μg plume than the naive males did in the 0.1-μg plume (0.42 vs 0.17 min, $F = 13.71$, $df = 113$).

E/Z11-14AL Plus Combinations of E11-14AC and SAT-14AL

To determine possible interactions caused by the presence of both E11-14AC and SAT-14AL, responses of males to septa loaded with E/Z11-14AL and various combinations of E11-14AC and SAT-14AL were recorded. The sequence of male behavioral steps was assayed in response to 0.1 μg E/Z11-14AL mixed with three concentrations of SAT-14AL (1, 3 and 10% of the concentrations of E/Z11-14AL) in combination with three concentrations of E11-14AC (10, 30 and 100% of the concentrations of SAT-14AL). These concentrations and combinations were chosen to bracket the proportions in which these compounds are found in the material emitted by calling females as reported by Silk et al. (1980). Assays were first carried out to determine the direct responses of naive males to the above combinations. Then, because activation levels with the 0.1-μg E/Z11-14AL were as low as in the last experiment, the assays were repeated with preactivated males.

Table 4. Responses of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with SAT-14AL loaded in rubber septa. Males were either exposed directly to the test chemicals (naive) or preactivated by exposure to a septum loaded with 1 μg E/Z11-14AL. The three E/Z11-14AL series were analyzed separately. Within each series, there were no significant differences.

		Naive males[a]							Preactivated males[a]					
E/Z11-14AL (μg)	SAT-14AL (as % of E/Z11-14AL)	n	LO (%)	30 cm (%)	SO (%)	COP (%)	INT	Flight speed (cm/sec)	n	30 cm (%)	SO (%)	COP (%)	INT	Flight speed (cm/sec)
0.01	0	20	0						20	5	100	100		
"	1	20	0						20	5	100	100		
"	3	20	0						20	20	100	100		
"	10	20	0						20	25	100	80		
"	30	20	0						20	15	100	100		
0.1	0	25	16	100	100	25			26	96	100	44	3.2	12.4
"	1	25	24	100	100	50			24	92	100	59	3.5	13.8
"	3	25	28	86	100	50			26	96	100	48	3.9	11.5
"	10	25	12	100	100	33			25	88	100	23	3.0	12.6
"	30	25	24	100	100	17			25	96	100	29	3.7	12.3
1.0	0	24	83	100	95	37	3.6	22.8						
"	1	24	88	100	100	33	4.3	18.0						
"	3	24	92	100	100	33	4.1	16.9						
"	10	24	96	100	100	22	4.2	18.1						
"	30	24	88	100	100	19	3.4	16.9						

[a] Percentages were calculated from the numbers that reached the previous behavioral stage. LO = locked on, 30 cm = came to within 30 cm of source, SO = reached source, COP = attempted copulation, INT = intensity of sexual activity (the number of 0.25-min intervals in a 3-min period in which wing fanning occurred).

As in the assays of septa containing E/Z11-14AL and SAT-14AL, the addition of 10% SAT-14AL resulted in a nonsignificant reduction in the numbers of naive males that locked onto the plume (Table 5). On average, 18.4% of the 25 males in each treatment proceeded upwind to the pheromone source and, of those that reached the source, 38% attempted copulation. There were, however, no differences attributable to the combinations of levels of SAT-14AL and E11-14AC. Among the males that were preactivated by the 1-μg E/Z11-14AL, an average of 96.7% proceeded upwind to the pheromone sources and, of those that reached the sources, 56.7% attempted copulation (Table 5); again, no differences could be attributed to the different combinations of SAT-14AL and E11-14AC.

As in Experiment 2, the preactivated males took significantly longer than the naive males to start moving upwind after the removal of the 1-μg septum (0.286 vs. 0.023 min; $F = 345.8$, df=229) but, once locked on, they proceeded upwind faster (0.046 vs 0.137 min; $F = 172.1$, df = 224) than the naive males, and attempted to copulate sooner (0.049 vs 0.120 min; $F = 18.5$, df = 109). However, there were no differences in flight times attributable to the different combinations of SAT-14AL and E11-14AC among either the naive or the preactivated males.

Finally, the lengths of time that males sustained flight in response to virgin females were compared with lengths of sustained flight in response to E/Z11-14AL in combination with SAT-14AL and E11-14AC. Female moths were housed, individually, in small screen cages. For the evaluation of sustained flight, two cages were fastened end to end to increase the probability of one female calling at any given time. Septa were loaded with 100 μg E/Z11-14AL alone and in combination with SAT-14AL and E11-14AC in the following combinations (expressed as % of the E/Z11-14AL concentration): a) 5% SAT-14AL, b) 50% SAT-14AL, c) 1% E11-14AC, d) 10% E11-14AC, e) 5% SAT-14AL and 1% E11-14AC, f) 50% SAT-14AL and 10% E11-14AC. These amounts approximate the natural blend and 10 times the concentration of the natural blend.

The duration of flight was recorded from the moment the male moth locked onto the pheromone plume until it veered out of the plume and landed on the inside of the tunnel. Previous observations had shown that males frequently sustained flight in plumes produced by virgin females for periods up to 1 hr. To avoid such time-consuming observations, all flights were terminated after 10 min.

Of 73 males that responded to the synthetic blends, only nine flew for more than 10 min, in comparison with eight of the 10 that responded to virgin females (Table 6). The average flight time of males that responded to females is therefore conservative, but even so, the average flight times and distances flown in response to the synthetic chemicals were all much shorter. Apparent speed, which is a calculation independent of flight duration, was also lower among males that responded to synthetic blends than among those that responded to females, but the differences were not significant because of high variability.

Table 5. Responses of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with SAT-14AL and E11-14AC in rubber septa. Males were either exposed directly (naive) or preactivated by exposure to a septum loaded with 1 μg E/Z11-14AL. No significant differences were detected.

E/Z11-14AL (μg)	SAT-14AL (as % E/Z11-14AL	E11-14AC (as % E/Z11-14AL	Naive males[a]					Preactivated[a]				
			n	LO (%)	30 cm (%)	SO (%)	COP (%)	n	30 cm (%)	SO (%)	COP (%)	INT
0.1	0	0	25	12	100	100	33	10	100	100	70	4.9
"	1	0	25	36	389	100	50	10	100	100	60	3.9
"	1	0.1	25	24	83	100	40	10	100	100	60	4.9
"	1	0.3	25	36	89	88	14	10	100	100	40	4.0
"	1	1.0	25	24	100	100	67	10	100	100	90	5.4
0.1	0	0	25	20	100	100	40	10	100	100	70	6.6
"	3	0	25	28	100	100	29	10	100	100	50	5.3
"	3	0.3	25	44	100	64	43	10	100	100	40	2.8
"	3	1	25	12	67	50	0	10	100	90	33	4.9
"	3	3	25	28	86	100	33	10	100	90	67	3.6
0.1	0	0	25	20	60	100	67	10	100	100	60	3.0
"	10	0	25	16	75	67	0	10	100	100	70	6.3
"	10	1	25	12	100	100	0	10	100	90	67	4.0
"	10	3	25	12	100	100	67	10	100	90	33	3.0
"	10	10	25	12	100	100	33	10	100	90	67	6.8

[a] Percentages were calculated from the numbers that reached the previous behavioral stage. LO = locked on, 30 cm = came to within 30 cm of source, SO = reached source, COP = attempted copulation, INT = intensity of sexual activity (the number of 0.25-min intervals in a 3-min period in which wing fanning occurred).

Table 6. Behavior of male spruce budworm during sustained flights in a wind tunnel in response to various combinations of E/Z11-14AL, SAT-14AL and E11-14AC, in comparison with responses to virgin females.

Loading in septa			Responses[a]				
E/Z11-14AL (μg)	SAT-14AL (as % of E/Z11-14AL)	E11-14AC (as % of E/Z11-14AL)	n	Number flying >10 min	Mean flight duration (min)	Apparent mean distance flown (m)	Apparent speed (cm/sec)
100	0	0	12	0	3.47 b	26.4 b	10.6
	5	0	11	2	3.76 b	30.7 b	10.3
	0	1	10	1	4.00 b	24.4 b	9.3
	5	1	11	1	4.70 b	38.8 b	12.0
	50	0	11	0	2.12 b	15.0 b	9.8
	0	10	9	3	5.50 ab	39.5 b	9.9
	50	10	9	2	4.52 b	36.4 b	10.5
2 virgin females			10	8	9.4 a	92.4 a	15.9

[a] Numbers in the same column followed by different letters are significantly different.

E/Z11-14AL Plus 16ALs

Behavior was also assayed in response to E/Z11-14AL plus either SAT-16AL, E11-16AL or Z11-16AL, dispensed from rubber septa. To determine if the 16ALs enhanced response, a low concentration of E/Z11-14AL (0.1 μg per septum) was used in each instance. All three 16ALs were assayed at concentrations of 0.3, 1, 3, and 10% of the concentration of E/Z11-14AL. Additional assays were carried out for 0.1, 1, and 10% SAT-14AL in combination with 1 and 10 μg of the E/Z11-14AL.

Results are shown in Tables 7, 8 and 9. The presence of SAT-16AL tended to reduce the number of males that locked onto the pheromone plumes and the proportion of males that flew upwind to the pheromone sources, although the differences were significant only with the 1.0-μg loadings of E/Z11-14AL (Table 7). Neither E11-16AL (Table 8) nor Z11-16AL (Table 9) had any significant effect on responses in comparison with E/Z11-14AL alone.

DISCUSSION

In previous investigations assays of male spruce budworm responses to suboptimal blends and concentrations of pheromone have been carried out by first activating the moths to a higher pheromone concentration (Sanders 1984, Silk and Kuenen 1986). This is a very effective assay technique because only the insects that respond to the initial higher concentration are included in the assay. This screens out variability as a result of day-to-day differences in the responsiveness of males. In the present experiment, males that initiated flight in response to a septum loaded with 1 μg E/Z11-14AL took significantly longer to lock onto the plume from a 0.1-μg septum than males exposed directly to a 0.1-μg septum without such preactivation. It is presumed that males adapted to the higher concentration and required several seconds to regain their responsiveness to the lower concentration. Casting flight is therefore clearly of advantage in providing the insect with the necessary time to disadapt before losing the plume. Once these preactivated males locked onto the lower-concentration plume, they flew upwind significantly faster than males that had not experienced the higher concentration previously. In so doing, they resembled males responding to a much lower pheromone concentration. Their perception of concentration would therefore appear to be influenced by change rather than by absolute concentration, which confirms previous conclusions (e.g., Kennedy et al. 1981).

The two main components of the spruce budworm sex pheromone are the E and Z isomers of 11-14AL in a ratio of approximately 95:5 (Sanders and Weatherston 1976, Silk et al. 1980, Alford et al. 1983, Sanders 1984). It has also been shown that the loading of this blend on filter paper that gave optimal responses is in the range from 0.1 to 10 μg, whereas the optimum loading in rubber septa is from 1 μg up to at least 1 mg, the highest loading tested (Sanders, manuscript in preparation).

Table 7. Responses of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with SAT-16AL loaded in rubber septa.

E/Z11-14AL (μg)	SAT-16AL (as % of E/Z11-14AL)	n	Proportion (%) of male moths that:[a] Locked on	Came within 30 cm of source	Reached source	Attempted copulation
0.1	0	25	24	100	100	50
"	0.3	25	36	100	100	22
"	1	25	24	100	100	0
"	3	25	20	100	100	20
"	10	25	16	100	75	0
1.0	0	55	98 a	96	90 ab	38
"	1	50	92 ab	100	91 a	43
"	1	25	76 b	95	67 ab	58
"	10	25	72 b	83	67 b	30
10	0	25	96	96	91	14
"	.1	25	76	84	88	36
"	1	25	84	81	71	33
"	10	25	76	63	50	17

Note: Numbers in the same column that are followed by different letters are significantly different (P = 0.05).

[a] Percentages were calculated from the numbers that reached the previous behavioral stage.

Table 8. Responses of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with E11-16AL loaded in rubber septa. (25 male moths used in each trial)

E/Z11-14AL (μg)	E11-16AL (as % of E/Z11-14AL)	Proportion (%) of male moths that:[a] Locked on	Came within 30 cm of source	Reached source	Attempted copulation
0.1	0	12	100	100	0
"	0.3	4	100	100	100
"	1	28	100	100	57
"	3	20	100	100	40
"	10	12	100	67	50

[a] Percentages were calculated from the numbers that reached the previous behavioral stage.

Table 9. Responses of male spruce budworm in a wind tunnel to E/Z11-14AL in combination with Z11-16AL loaded in rubber septa.

E/Z11-14AL (μg)	Z11-16AL (as % of E/Z11-14AL)	n	Proportion (%) of male moths that:[a] Locked on	Came within 30 cm of source	Reached source	Attempted copulation
0.1	0	52	27	100	93	42
"	0.3	25	16	100	100	25
"	1	53	21	82	100	43
"	3	48	23	91	100	43
"	10	48	21	100	90	20
1.0	0	10	70	100	100	NR[b]
"	1	10	90	100	100	NR
"	3	6	83	100	90	NR
"	10	5	80	75	100	NR

[a] Percentages were calculated from the numbers that reached the previous behavioral stage.

[b] NR = not recorded

Recent work by Silk and Kuenen (1988) has implicated the presence of 16-carbon-chain aldehydes in the pheromone system of the spruce budworm. However, the results of the present experiments indicate no effect on male response with the addition of either E or Z11-16AL to E/Z11-14AL, and a slight reduction in response with the addition of SAT-16AL.

The addition of the acetate E11-14AC to the basic 95:5 E/Z11-14AL mixture at concentrations of 10% or higher caused reductions in response, both with filter-paper dispensers, as reported previously (Alford et al.

1983), and with rubber septa. This confirms earlier observations (Sanders 1976, 1984), but also establishes for the first time that the presence of the E11-14AC suppresses all steps in the behavioral sequence, including changes in the speed of flight.

The addition of SAT-14AL, alone or in combination with E11-14AC, produced no significant effect on any of the responses measured in these assays. Effects have been reported in previous studies, but the results have not been entirely consistent. Alford et al. (1983) claimed that the addition of SAT-14AL increased the numbers of male spruce budworm that contacted a lure in a wind tunnel, although their data do not support this conclusion (their Table 1). In their field studies, Alford et al. (1983) found that the addition of 1% SAT-14AL had no effect on the number of insects that approached a pheromone source. However, this addition **decreased** significantly the proportion of activated males that came to within 7.5 cm of the source, but significantly **increased** the proportion that contacted the source. The addition of 5% SAT-14AL increased the proportion that approached the source (but only from 75 to 82%), whereas the addition of either 1% or 5% SAT-14AL resulted in significantly higher proportions of male moths that contacted the source and in longer periods of contact.

Silk and Kuenen (1986) showed that the addition of 5% SAT-14AL significantly increased the number of males that contacted a septum loaded with 30 ng E/Z11-14AL, but did not increase the number attracted to septa loaded with 10 or 100 ng. In wind tunnel experiments, Sanders (1984) found that the addition of 2% SAT-14AL to E/Z11-14AL in PVC lures loaded with 0.03% pheromone (weight/weight) significantly increased the numbers of males that contacted the lures. In addition it offset the 'inhibitory' effect of E11-14AC, as also reported by Silk et al. (1980). Sanders (1984) also found that the numbers of males that began wing fanning, that locked onto the pheromone plume, and that flew upwind in response to the synthetic blends often equaled the level of response to virgin females, but the males always flew faster and persisted longer in upwind flight in response to calling female moths. In a subsequent report (Sanders 1985) it was stated that additions of 1, 10 and 100 μg SAT-14AL to 100 μg E/Z11-14AL in rubber septa significantly increased the percentage of males that attempted to copulate. However, when the percentages are calculated on the basis of the number of males that reached the source, rather than on the total number of males assayed, the differences are not significant. Grant (1987) found that males attempted copulation with rubber septa sooner and persisted in their attempts longer in response to a 95:5:2 blend than to the 95:5 blend alone.

Initially these same three parameters (number that contacted the source, latency of copulation, and persistence) were measured in the present study, but measurements were discontinued. It proved difficult to determine when contact was broken, and to decide how to score insects that left the source but returned. Similarly, decisions on the number of copulatory attempts became very subjective, because results depended on

when one attempt stopped and the next began. Measures of latency were discontinued when it was found that they were too variable. In place of these a new, less subjective parameter, the intensity of sexual activity, was devised. Significant differences in intensity were found as a result of the different concentrations of E/Z11-14AL alone (Table 2), but no differences could be ascribed to the presence of E/Z11-14AL or E11-14AC alone or in combination (Tables 2, 4 and 5).

Clearly, the effects of SAT-14AL on in-flight behavior are, if real, very subtle. It is possible that larger samples in the present study would have uncovered significant differences. However, it is questionable if such minor differences are of biological significance and they certainly do not explain the wide differences between male in-flight behavior in response to synthetics and to virgin female moths.

SUMMARY AND CONCLUSION

- This study failed to demonstrate any enhancement of male spruce budworm responses to the main pheromone components (E/Z11-14AL) by the addition of two identified minor components, SAT-14AL and E11-14AC, either alone or in combination.

- The study also showed no enhancement of response by the addition of the 16ALs, SAT-16AL, E11-16AL or Z11-16AL.

- Although previous studies have shown enhancement of some behavior by SAT-14AL, the results have been inconsistent and levels of response have never reached those evoked by the female-emitted pheromone.

- It is therefore concluded that there are additional unidentified pheromone components.

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